UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: April 17, 2013

SUBJECT: Bicyclopyrone: Summary of Hazard and Science Policy Council (HASPOC) Meeting of March 14, 2013: Recommendations on the Requirement of the Developmental Neurotoxicity Screening Study.

PC Code: 018986
Decision No.: N/A
Petition No.: N/A
Risk Assessment Type: N/A
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MRID No.: N/A

DP Barcode: N/A
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THROUGH: Jess Rowland, Co-Chair
Anna Lowit, Ph.D., Co-Chair
HASPOC
HED (7509P)

TO: Anwar Y. Dunbar, Ph.D.
Dana M. Vogel, Deputy Director
Risk Assessment Branch 1 (RAB1)
HED (7509P)

MEETING ATTENDEES:

HASPOC Members: Anna Lowit, Elissa Reaves, Elizabeth Mendez, Jess Rowland, John Kough, Jonathan Chen, Michael Metzger, Ray Kent, Charles Smith for Jeff Evans

Presenter: Anwar Dunbar

Other Attendees: Jaime D'Agostino, Monique Perron, Joey Bever, Angela Howard, Matt Lloyd, Jennifer Tyler, Chris Schlosser, Kristin Rury, Julie Van Alstine

I. PURPOSE OF MEETING:

Risk Assessment Branch 1 (RAB1) is working on a global joint review for bicyclopyrone. Bicyclopyrone is a new herbicide generated by Syngenta for a proposed use on corn. Syngenta has submitted a waiver request for a developmental neurotoxicity (DNT) study (MRID No. 48932902). At the request of the RAB1 risk assessment team, the HASPOC met on March 14, 2013 to discuss the registrant's request to waive the requirement for this study.

The petitioner, Syngenta, has previously given two presentations to the Agency regarding the toxicity studies for bicyclopyrone (July 13, 2011 and June 26, 2012). The purpose of the 2011 meeting was to: 1) summarize Syngenta's findings from the developmental and reproductive studies; 2) to address the effects in the ganglion in the dog (discussed below); and 3) to receive OPP's opinion on the usefulness of conducting a DNT study in rats. The purpose of the 2012 meeting with Syngenta was to report the preliminary findings and difficulties experienced with attempts to conduct a DNT study in Sprague-Dawley and Wistar rats.

II. WEIGHT OF EVIDENCE APPROACH:

Developmental Neurotoxicity Study

According to the revised 40 CFR Part 158 Toxicology Data Requirements, a DNT study is a "Conditional Requirement" and will be required using a weight of evidence (WOE) approach. Therefore, HASPOC used the following factors in the WOE approach:

- **Toxicological profile and mode of action:** Bicyclopyrone is a second generation inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD) and belongs to a class of herbicides that include pyrasulfotole, mesotrione, and isoxaflutole. Inhibition of HPPD eventually results in impairment of chlorophyll biosynthesis and tyrosinemia in certain mammals. The target organs for this class are the eye, liver, and kidney. The ocular toxicity in rats occurs due to the inhibition of tyrosine metabolism causing tyrosinemia. In 2001, HED's Metabolism Toxicity Assessment Committee (MTARC) concluded that for tyrosine-mediated toxicological effects, the mouse is a more appropriate model for assessing human risk than is the rat (TXR No. 0051908). This decision is based on comparative data on the activity of tyrosine aminotransferase (TAT) in the rat, mouse, and human, and the similarities of the response to elevated plasma tyrosine levels in humans and the mouse.

- **Evidence for neurotoxicity in the bicyclopyrone database of toxicology studies:** In the chronic dog study at dose levels of 2.5, 25, or 125 mg/kg, one male from the top dose group was found dead after 336 days; there had been no prior clinical signs. Signs of toxicity included corneal opacity and keratitis in some males and females at 25 and 125 mg/kg. Histopathological changes in the nervous system, chromatolysis, swelling of selected neurons in the dorsal root ganglion, and minimal fiber degeneration in the sciatic nerve were observed in some dogs from all dosed groups. These findings in the dorsal root ganglion were not associated with any signs of neurological dysfunction and were not seen in association with other findings in the peripheral nervous system or in related

areas of the central nervous system, suggesting that these changes are not of toxicological significance.

In the acute neurotoxicity study for bicyclopyrone, transient effects on motor activity were noted at 2000 mg/kg for the first 20 minutes post dose only. In the control and 2000 mg/kg groups, axonal degeneration was noted in spinal root fibers and peripheral nerves but was of minimal severity in all instances and was considered spontaneous. Spontaneous axonal degeneration has been reported as the most frequent lesion in the central and peripheral nervous systems of both control and treated rats (Eisenbrandt *et al.*, 1990).

In the subchronic neurotoxicity study [0, 4, 35, and 336 mg/kg/day (males) and 0, 4, 42, and 415 mg/kg/day (females)], there was no treatment related effect on functional-observation battery (FOB) or neuropathology at any dose level tested. Slightly lower group mean brain weight was noted in males (≤ 12%) only at the highest dose tested (336 mg/kg/day), but the relationship to treatment was considered equivocal because of the absence of any difference in females, the absence of any adverse neuropathological findings, and the unusually high group mean brain weight in concurrent control males.

In summary, the current data suggest that bicyclopyrone caused neurotoxicity in rats only at high doses which are well above the doses that causes target organ toxicity (eyes, kidney, and liver) in a more sensitive species (dogs).

- **Evidence for fetal and offspring toxicity in the bicyclopyrone database:** In two of the prenatal developmental toxicity studies in rabbits, there was an increased quantitative fetal susceptibility which manifested as skeletal variations and abnormal urogenital development. In the other rabbit and rat developmental studies, fetal and maternal effects occurred at the same dose. Fetal effects consisted of decreased body weights, and skeletal abnormalities. Increased post-implantation loss and abnormal cardiac development were observed at higher doses. Maternal effects consisted of decreased body weights, body weight gains, food consumptions, decreased activities, and macroscopic findings in the stomach.

  In the two-generation reproduction study for bicyclopyrone, the effects in offspring included decreased body weight gain and ocular and kidney effects which were dose-dependent (500 and 5000 ppm). Parental effects consisted of ocular toxicity at all doses tested, decreased body weight gains at higher doses, and changes in the weights of the liver, kidney, and thyroid.

- **DNTs performed for other members of this class:** Since the rat is an inappropriate species for HPPD inhibitors, EPA had previously required a DNT study in mice as part of conditional registration of mesotrione (TXR No. 014536). The registrant conducted several studies and modified the experimental techniques to conduct the DNT study in mice. The registrant also conducted several studies using a positive control (to demonstrate their ability to conduct the DNT study in mice) and established the baseline data set required to evaluate the results of the mesotrione DNT study in mice (since most

of the DNT studies are conducted in rats). These results were submitted to and reviewed by HED (TXR No. 0053700 and 0053425). The review of these results clearly demonstrated the difficulties with producing sound positive controls in mice, compromising any outcome of a DNT study in this species. Therefore, HED concluded that the mouse was not a viable model for DNT.

- **Preliminary DNT studies:** The Registrant conducted three preliminary studies to determine the doses for the main study.

  In the first study, groups of mated female Sprague-Dawley rats were provided diets containing NOA449280 at target concentrations of 0 (control), 25, 500, and 5000 ppm beginning on day 6 of gestation and continuing through day 21 of lactation (Beck 2012c). These dose levels were the same as those used in the two-generation reproduction study in the Wistar rat. Based on the findings from this study, NOA449280 dietary concentrations of 25, 500, and 5000 ppm were selected for use in a guideline developmental neurotoxicity study to be conducted using Sprague-Dawley rats. Due to the unexpected high mortality observed in the Sprague-Dawley rat in the DNT study compared to both the two-generation study in Wistar rats and the preliminary study in Sprague-Dawley rats, a repeat preliminary study was conducted.

  In the second study, groups of bred Sprague-Dawley female rats were provided diets containing 0, 100, 500, or 5000 ppm NOA449280 beginning on gestation day 6 through the termination of the study (Toot 2012b). Dose levels of 500 and 5000 ppm were selected to attempt to confirm the effects on survival observed in the guideline study, while a low dose of 100 ppm was selected to maximize the likelihood of observing any effect on survival given the small group size in this preliminary study. There were no effects on litter size or the number or percentage of pups born alive in any dose group, which was also consistent with the lack of effects in both the initial preliminary study and the terminated guideline study. Early postnatal survival was significantly affected at 500 and 5000 ppm during the first postnatal day (PND 0-1). Survival was reduced on PND 1-4 in all dose groups. Overall survival from birth through PND 4 was statistically significantly decreased at all dose levels, though not to the extent observed in the terminated DNT study. The differences observed in survival rates were attributed to variability due to the smaller group size (10/dose) in this study compared to the terminated DNT study (30/dose).

  In order to determine whether the difference observed in pup survival between the Sprague-Dawley and Wistar rats was a reflection of a true strain difference or secondary to differences between the laboratories used for the various studies involving littering (multigeneration reproduction study or DNT), a comparative study was conducted (Toot 2012c). Groups of mated female Wistar rats were provided diets containing NOA449280 at concentrations of 0, 100, 500, or 5000 ppm. For comparative purposes, groups of mated female Sprague-Dawley rats were provided diets containing 0 or 500 ppm NOA449280.

Results from these studies and one abbreviated guideline DNT study with NOA449280 in the rat support the conclusions that a DNT study would not contribute useful data for human risk assessment and that this study requirement be waived.

In summary, adverse effects seen in the rat following administration of NOA449280 are attributable to the inhibition of HPPD and the consequent elevation of plasma tyrosine concentrations. There is no evidence for an additional mode of action (MOA) and no evidence of neurotoxicity in the rat. The toxicology database with NOA449280 does not demonstrate any clear evidence of neurotoxicity. .

III. HASPOC CONCLUSIONS:

The HASPOC, using a WOE approach, concluded that a DNT is not required for bicyclopyrone at this time based on the following considerations: 1) the relatively low evidence of neurotoxicity in the bicyclopyrone database; 2) the observed prenatal sensitivity does not involve the nervous system, and there was demonstrated decreased pup viability in two attempts to perform a DNT in rats; 3) the lack of a neurotoxic mechanism of action for this active ingredient; and 4) the demonstrated difficulties performing this study for other members of this class (mesotrione).